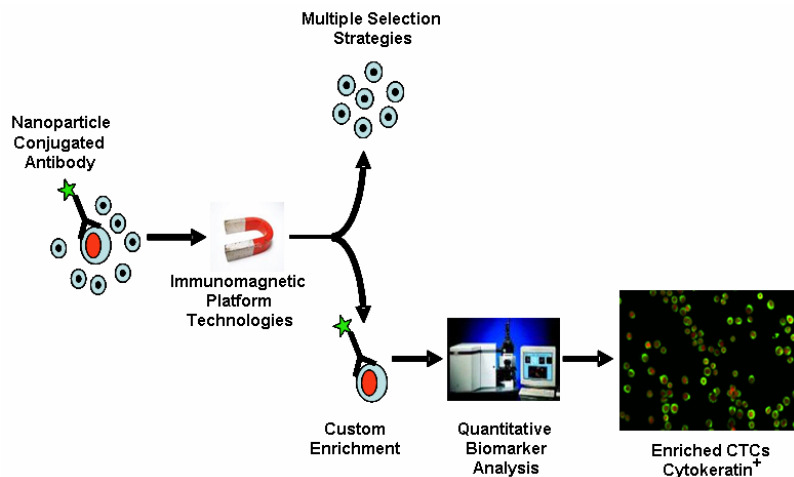
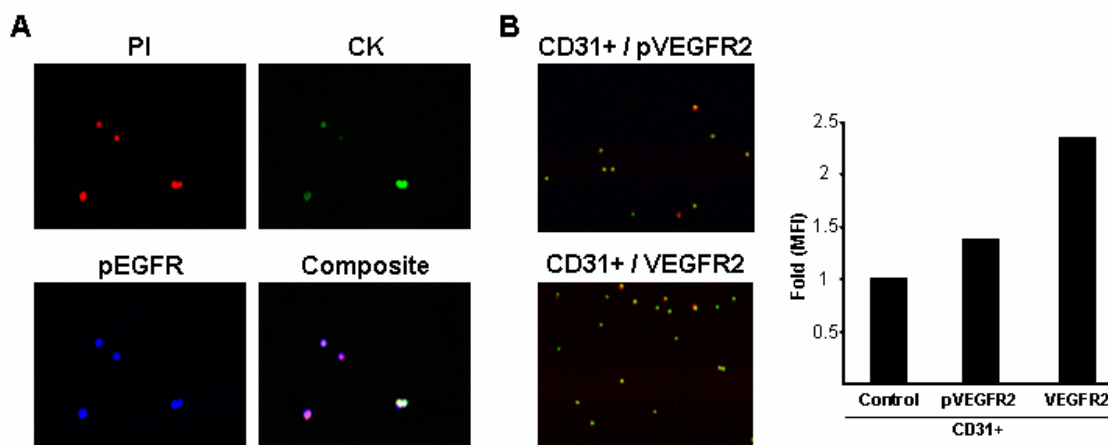


Case Study #5 - Development of Biomarker Assays for Monitoring Target Inhibition in Circulating Tumor Cells and Circulating Endothelial Cells

Monitoring pharmacodynamic modulation of drug effects in clinical studies can have profound consequences for successful drug development and ultimately regulatory approval. ApoCell has pioneered quantitative based assays for measuring cell-specific target inhibition, cell signaling and apoptosis using *in vitro* cell systems, tumor biopsies, and surrogate specimens including skin biopsies. Although biopsies can provide comprehensive pharmacodynamic information, they can be difficult to obtain and very costly. Scientists at ApoCell have developed an innovative method for quantifying biomarkers in circulating tumor cells (CTCs) and circulating endothelial cells (CECs). The results show the feasibility to perform biomarker expression analysis on rare cells enriched from peripheral blood.



CTCs and CECs were isolated from peripheral human blood using an optimized immunomagnetic enrichment method for EpCAM⁺ and CD31⁺ cells, respectively. Following enrichment, cells were fixed onto slides using a proprietary process and immunofluorescently stained for phosphorylated EGFR (pEGFR) or phosphorylated VEGFR2 (pVEGFR2) and total VEGFR2. Cells were scanned for biomarker expression using laser scanning technology.



A. pEGFR expression in colon cancer cells enriched from spiked human blood. Tumor cells were enriched using EpCAM⁺ immunomagnetic nanoparticles and immunofluorescently stained for Cytokeratin (green), pEGFR (blue), and nuclei (red). Laser generated images were captured for each biomarker and the composite images show co-localization of cytokeratin, pEGFR, and nuclei (white). **B.** Laser generated images of endothelial cells enriched using CD31⁺ immunomagnetic nanoparticles and immunofluorescently stained for pVEGFR2⁺ or VEGFR2⁺ cells (red), and nuclei (green). Composite images show co-localization of pVEGFR2 or VEGFR2 and nuclei (yellow). The mean fluorescent intensity of pVEGFR2 and VEGFR on CD31⁺ cells is shown relative to the negative control.

These results demonstrate ApoCell's proprietary capability to isolate phenotype-specific cells from human peripheral blood and analyze important drug targets such as EGFR and VEGFR2. ApoCell's integrated platform technologies are particularly useful for pharmacodynamic analysis on drug targets when tissue biopsies are limited.

Reference

1. Clinical Biomarker Summit, Abstract #1, March 19-21, 2007, Coronado, California